



ProFoldin

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INSTRUCTIONS

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SARS-CoV-2 RNA Polymerase (RdRp) Assay Kits

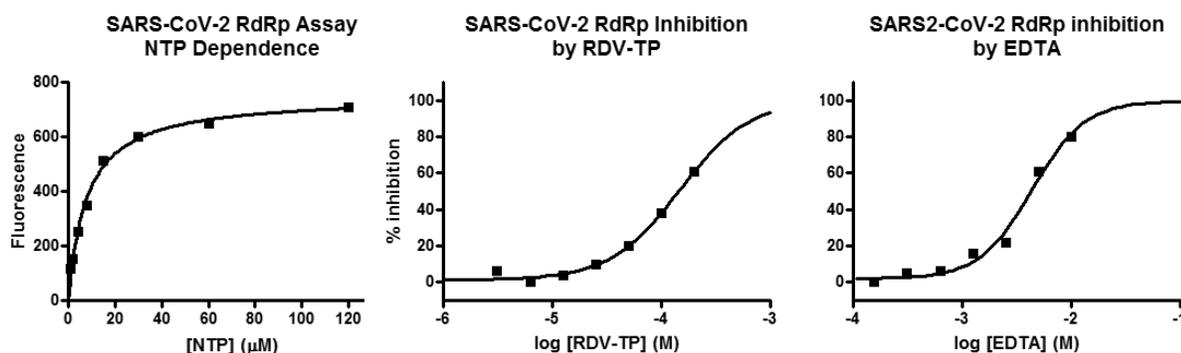
SARS-CoV-2 RNA polymerase (RdRp) assay kit-100
SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -20
SARS-CoV-2 RNA polymerase (RdRp) assay kit plus -100

Catalog No. S2RPA100K
Catalog No. S2RPA020KE
Catalog No. S2RPA100KE

Introduction

The COVID-19 pandemic is caused by infection of SARS-CoV-2 or severe acute respiratory syndrome coronavirus 2, a positive-sense single-stranded RNA virus. The RNA dependent RNA polymerase (RdRp) is a key drug target for this deadly virus. The high throughput SARS-CoV-2 RdRP assay is based on detection of the RNA molecules synthesized by the RNA polymerase in the presence of an RNA template and NTPs. The assay can be performed in a 384-well or 96-well plate format for tests of the enzyme activities of the COVID-19 virus RNA polymerase (SARS-CoV-2 RdRp, the complex of nsp12, nsp7 and nsp8 proteins) and high throughput screening of inhibitors.

The gel-based assay is also available for visualization of the RNA product synthesized by RdRp and inhibition by inhibitors (<https://www.profoldin.com/covid-19-drug-targets.html>).



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SARS-CoV-2 RNA polymerase (RdRp) assay kit-100 (Catalog No. S2RPA100K) includes 300 μl of 10 x Buffer, 50 μl of 50 x RNA template, 60 μl of 50 x NTPs (ATP, UTP, GTP, CTP) and 700 μl of 10 x fluorescence dye. It is for 100 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 50 assays in a 96-well plate format. The assay kit includes all reagents except the enzyme.

SARS-CoV-2 RNA polymerase (RdRp) assay kit plus-100 (Catalog No. S2RPA100KE) includes 300 μl of 10 x Buffer, 50 μl of 50 x RNA template, 60 μl of 50 x NTPs (ATP, UTP, GTP, CTP), 700 μl of 10 x fluorescence dye and 50 μl of 50 x SARS-CoV-2 RdRp. It is for 100 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 50 assays in a 96-well plate format.



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SARS-CoV-2 RNA polymerase (RdRp) assay kit plus-20 (Catalog No. S2RPA020KE) includes 75 µl of 10 x Buffer, 10 µl of 50 x RNA template, 12 µl of 50 x NTPs (ATP, UTP, GTP, CTP), 150 µl of 10 x fluorescence dye and 10 µl of 50 x SARS-CoV-2 RdRp. It is for 20 assays of SARS-CoV-2 RNA polymerase in a 384-well plate format or 10 assays in a 96-well plate format.

Assay Protocol for Enzyme Activity

The following assay protocol is based on the 384-well plate assay format (plate type: Matrix 4318 or alike). The reaction volume is 25 µl and the final assay volume is 90 µl. For 96-well plate assays (plate type: Costar 3915 or alike), the reaction volume is 50 µl and the final assay volume is 180 µl.

- Reaction:** The total volume of each reaction mixture is 25 µl including 21 µl of H₂O, 2.5 µl of 10 x Buffer, 0.5 µl of 50 x template, 0.5 µl of 50 x RNA polymerase and 0.5 µl of 50 x NTPs. Incubate the reaction mixture at 37°C for 2 hours.
- Detection:** Dilute the 10x fluorescence dye with water to make the 1 x dye. Mix 65 µl of the 1 x dye with 25 µl of the reaction mixture. Measure the fluorescence intensity at 535 nm using the excitation wavelength at 485 nm in 5 min.

Assay Protocol for Enzyme Inhibition

For the SARS-CoV-2 RdRp assay in a 96-well plate format:

- Add 1 µl of compound in DMSO into each well of the 96-well assay plate.
- Add 48 µl of a premix composed of 41 µl of H₂O, 5 µl of 10 x Buffer, 1 µl of 50 x Template and 1 µl of 50 x RdRp. After 5 min, add 1 µl 50 x NTP and Incubate the reaction at 37 °C for 120 min.
- Add 130 µl of 1x dye and read the fluorescence in 5 min.

Reference:

- Pacl H. T et al, Water-soluble tocopherol derivatives inhibit SARS-CoV-2 RNA-dependent RNA polymerase. bioRxiv, Cold Spring Harbor Laboratory, July 27, 2021.
- Goc A. et al, Simultaneous Inhibition of SARS-CoV-2 Infectivity by a Specific Combination of Plant-derived Compounds. European Journal of Biology and Biotechnology, Vol 2, Issue 5, p25 (2021).
- Goc A. et al, Inhibitory effects of specific combination of natural compounds against SARS-CoV-2 and its Alpha, Beta, Gamma, Delta, Kappa, and Mu variants. European Journal of Microbiology and Immunology, 21 Jan 2022.

Related Products – Other RNA polymerase and DNA polymerase assays

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|-------------|---|
| GS2RPA020KE | Gel-based SARS-CoV-2 RdRp assay kit |
| VRT100K | Viral (Flavivirus) RNA-dependent RNA Polymerase Assay Kit - 100 |
| RPA100KE | <i>E. coli</i> RNA Polymerase Assay Kit Plus |
| MRPA100KE | Human Mitochondrial RNA Polymerase Assay Kit Plus |
| T7RPA100KE | T7 RNA Polymerase Assay Kit Plus |
| HDPA100KE1 | Human DNA Polymerase Alpha Assay Kit Plus |
| HIV100KE | HIV Reverse Transcriptase Assay Kit Plus |

For more information of drug targets and enzyme assays, please visit www.profoldin.com.